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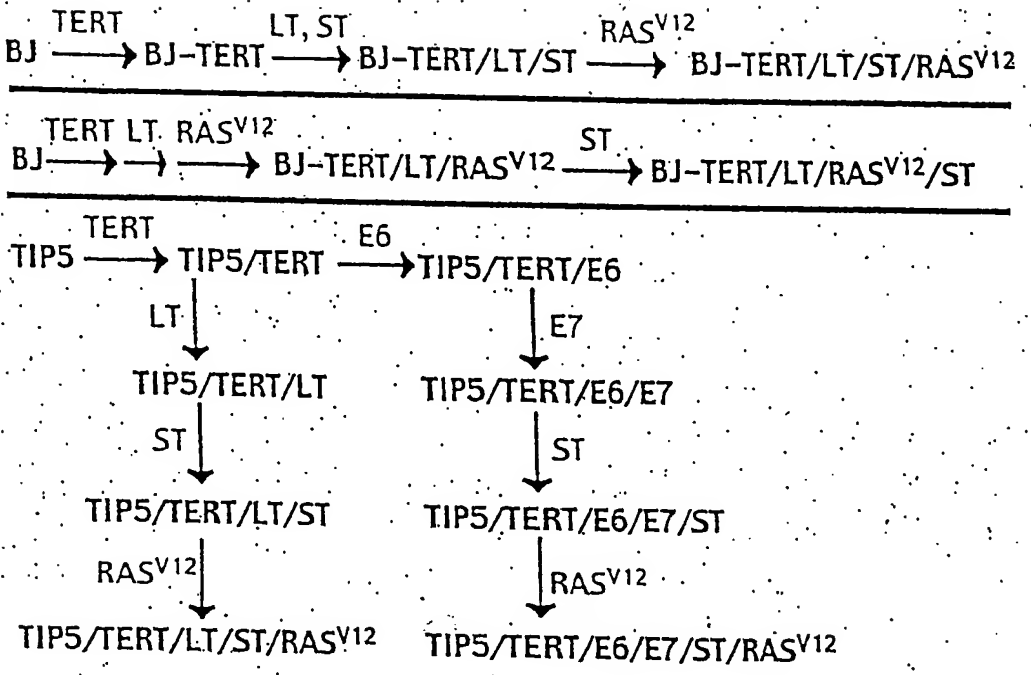
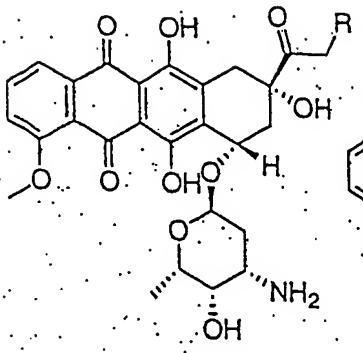
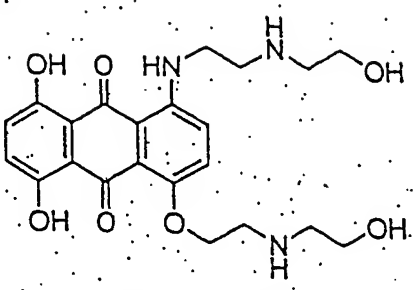

BJ
TERT
BJ-TERT
LT, ST
BJ-TERT/LT/ST
RAS^{V12}
BJ-TERT/LT/ST/RAS^{V12}
BJ
TERT LT RAS^{V12}
BJ-TERT/LT/RAS^{V12}
ST
BJ-TERT/LT/RAS^{V12}/ST
TIP5
TERT
TIP5/TERT
E6
TIP5/TERT/E6
LT
E7
TIP5/TERT/LT
TIP5/TERT/E6/E7
ST
ST
TIP5/TERT/LT/ST
TIP5/TERT/E6/E7/ST
RAS^{V12}
RAS^{V12}
TIP5/TERT/LT/ST/RAS^{V12}
TIP5/TERT/E6/E7/ST/RAS^{V12}

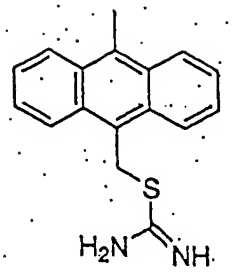
Figure 1



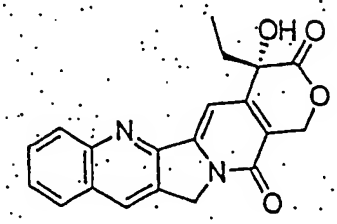
R= H, daunorubicin
R = OH, doxorubicin



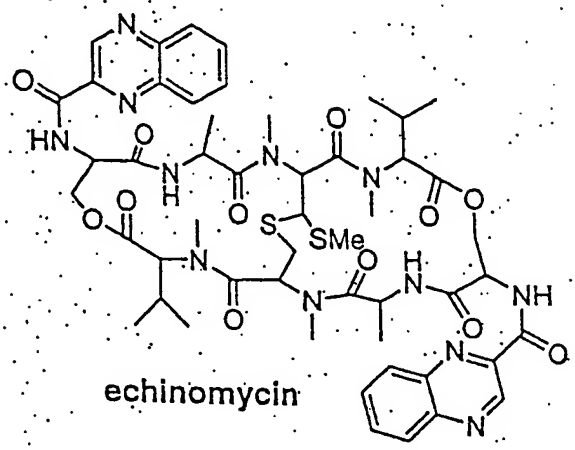
mitoxantrone



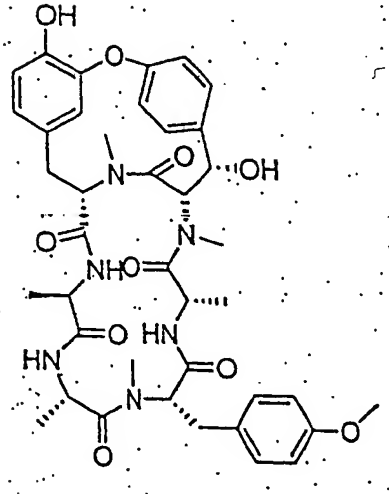
NSC146109



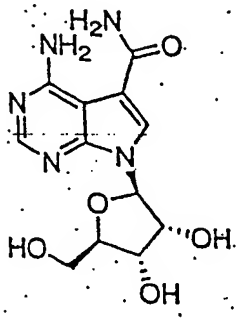
camptothecin



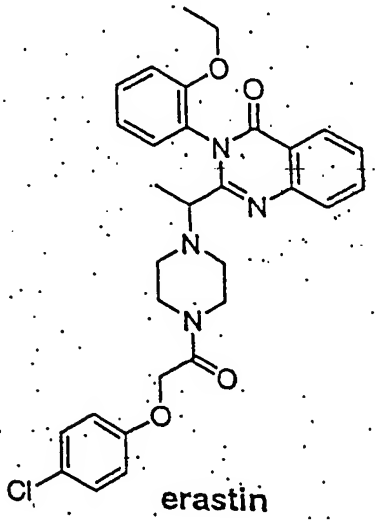
echinomycin



bouvardin



sangivamycin



erastin

Figure 2

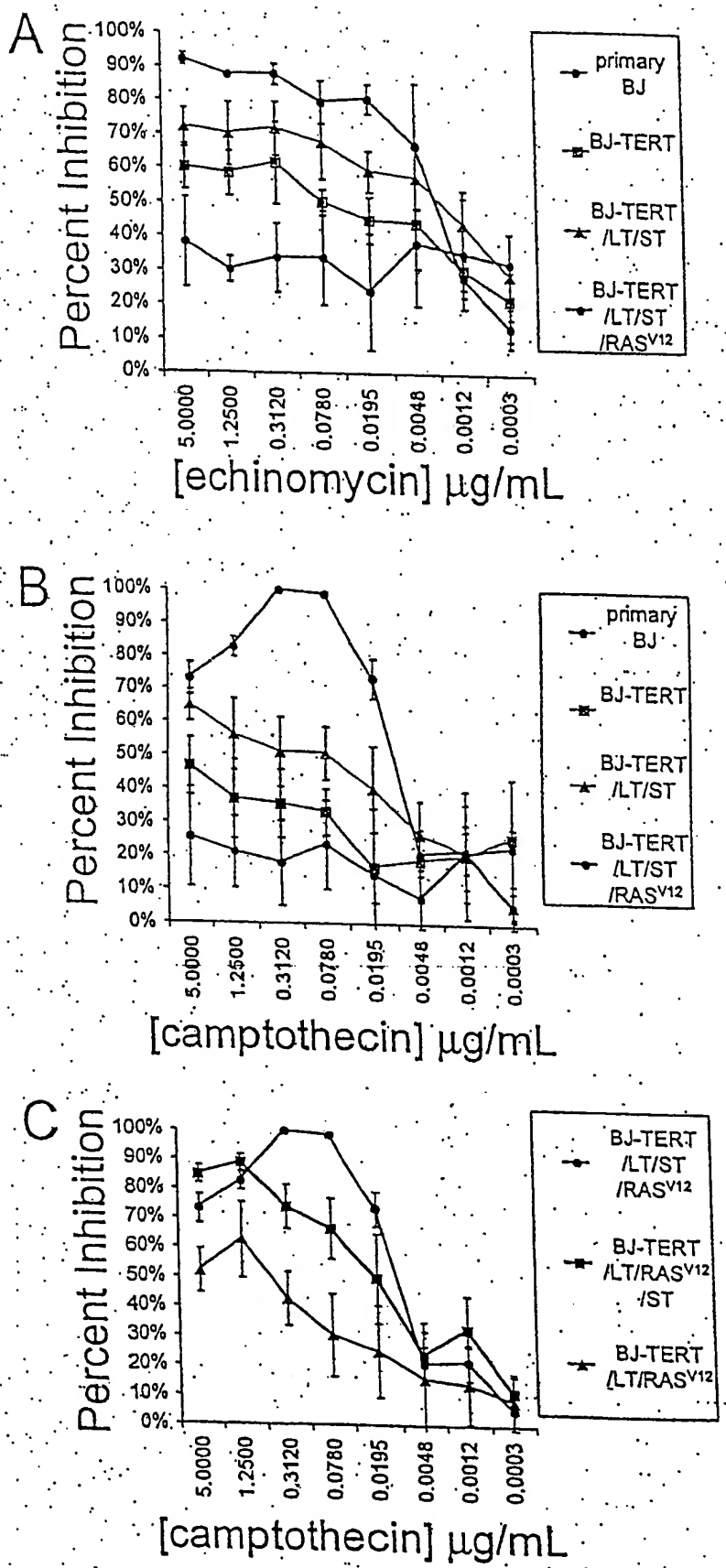

A
Percent Inhibition
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5.0000
1.2500
0.3120
0.0780
0.0195
0.0048
0.0012
0.0003
[echinomycin] µg/mL
primary BJ
BJ-TERT
BJ-TERT /LT/ST
BJ-TERT /LT/ST /RASV12
B
Percent Inhibition
[camptothecin] µg/mL
primary BJ
BJ-TERT
BJ-TERT /LT/ST
BJ-TERT /LT/ST /RASV12
C
Percent Inhibition
[camptothecin] µg/mL
BJ-TERT /LT/ST /RASV12
BJ-TERT /LT/RASV12 /ST
BJ-TERT /LT/RASV12

Figure 3

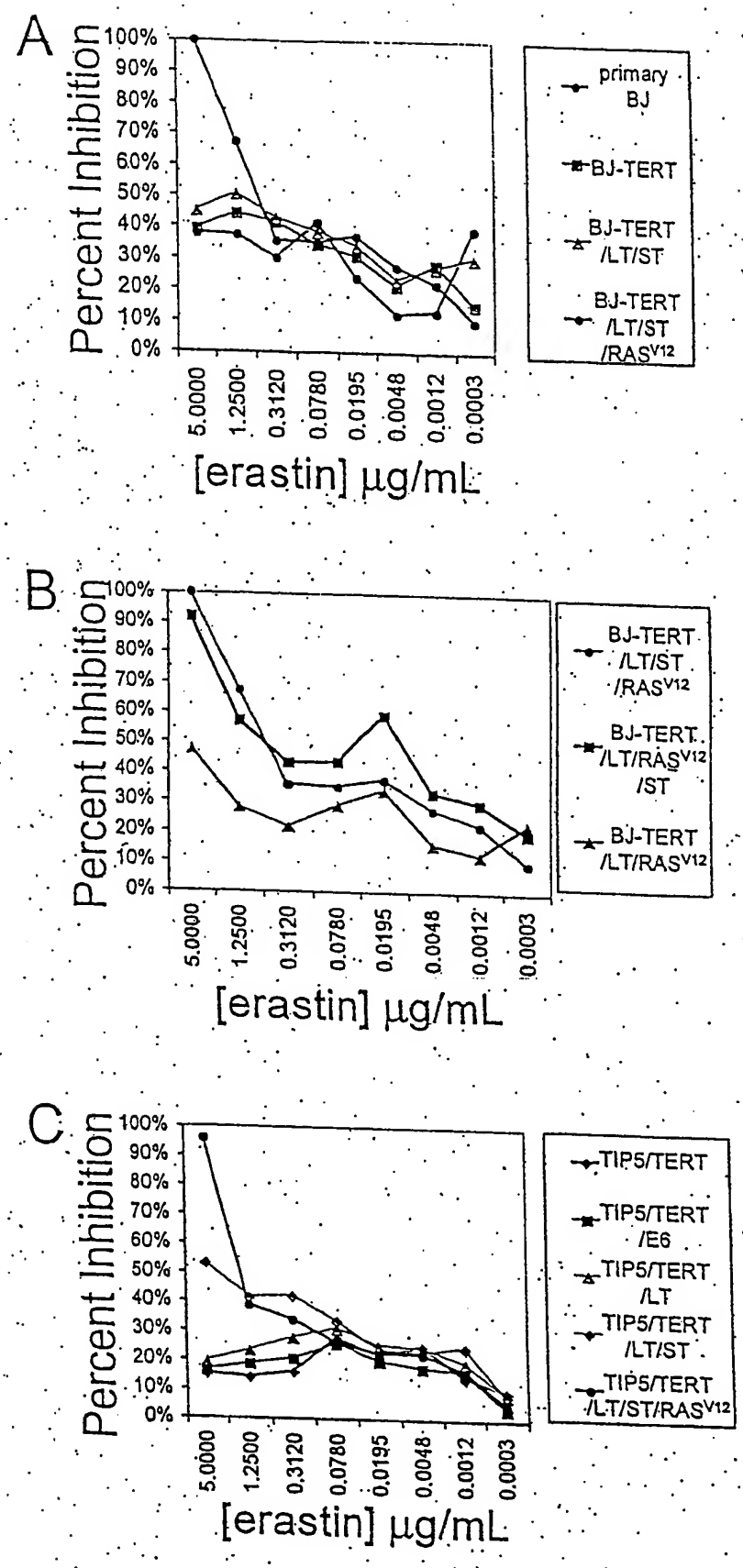

A
Percent Inhibition
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
5.0000
1.2500
0.3120
0.0780
0.0195
0.0048
0.0012
0.0003
[erastin] µg/mL
primary BJ
BJ-TERT
BJ-TERT /LT/ST
BJ-TERT /LT/ST /RASV12
B
Percent Inhibition
[erastin] µg/mL
BJ-TERT /LT/ST /RASV12
BJ-TERT /LT/RASV12 /ST
BJ-TERT /LT/RASV12
C
Percent Inhibition
[erastin] µg/mL
TIP5/TERT
TIP5/TERT /E6
TIP5/TERT /LT
TIP5/TERT /LT/ST
TIP5/TERT /LT/ST/RASV12

Figure 4

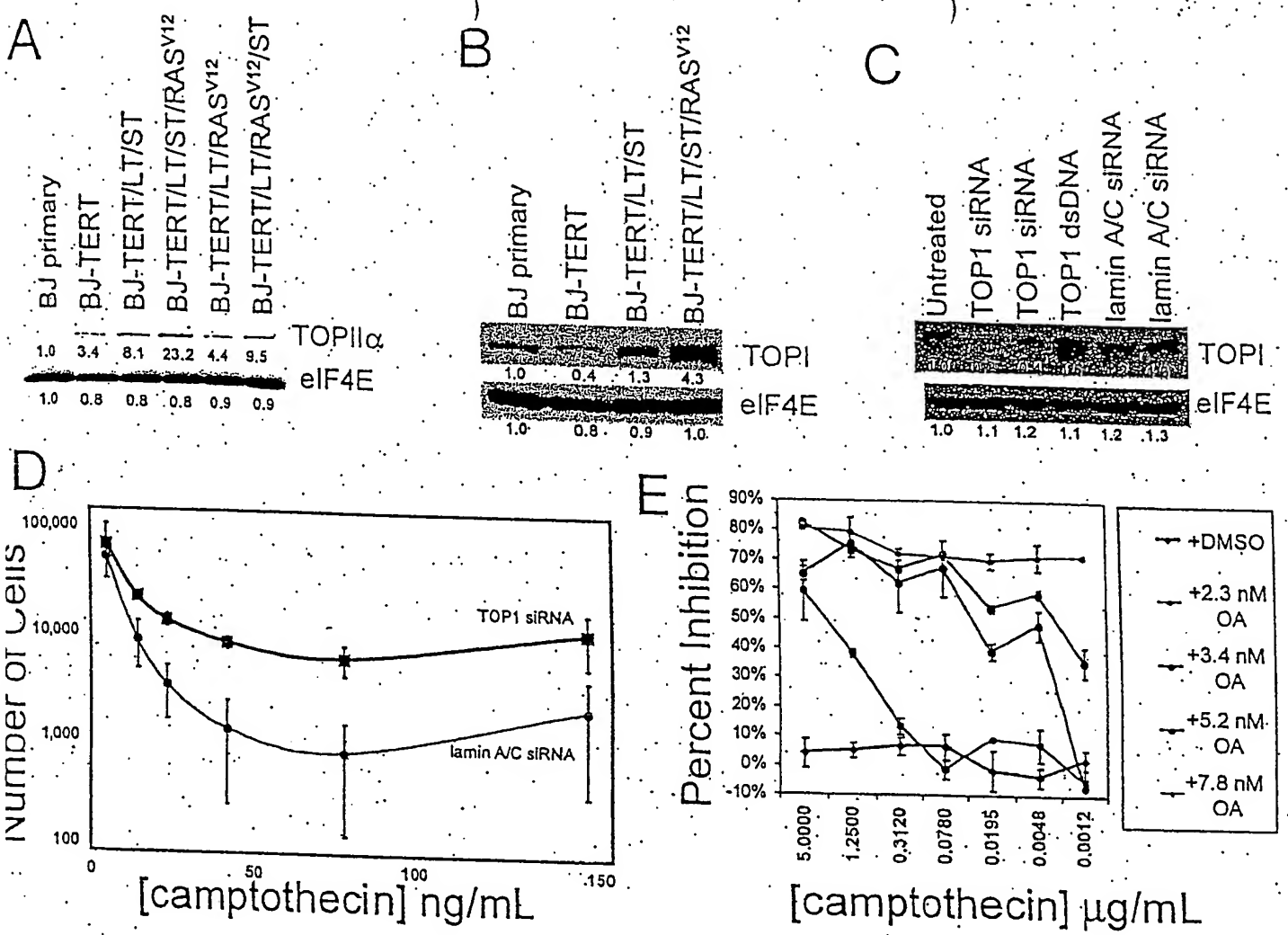
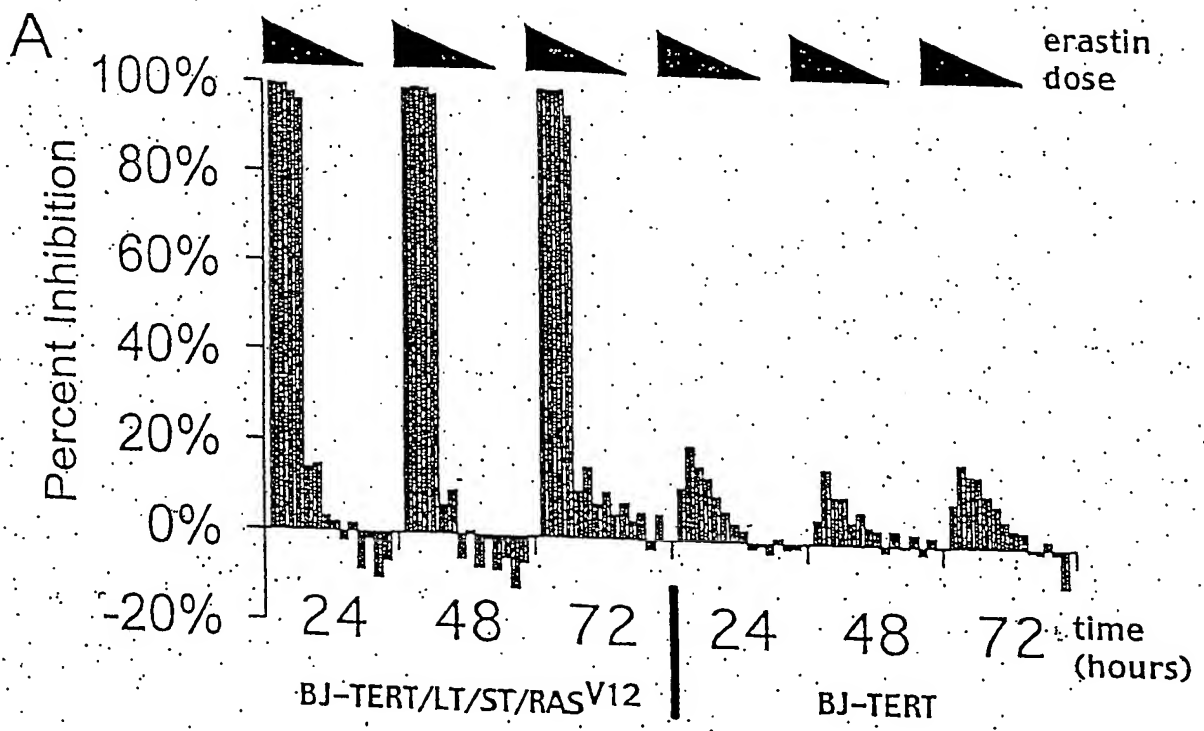
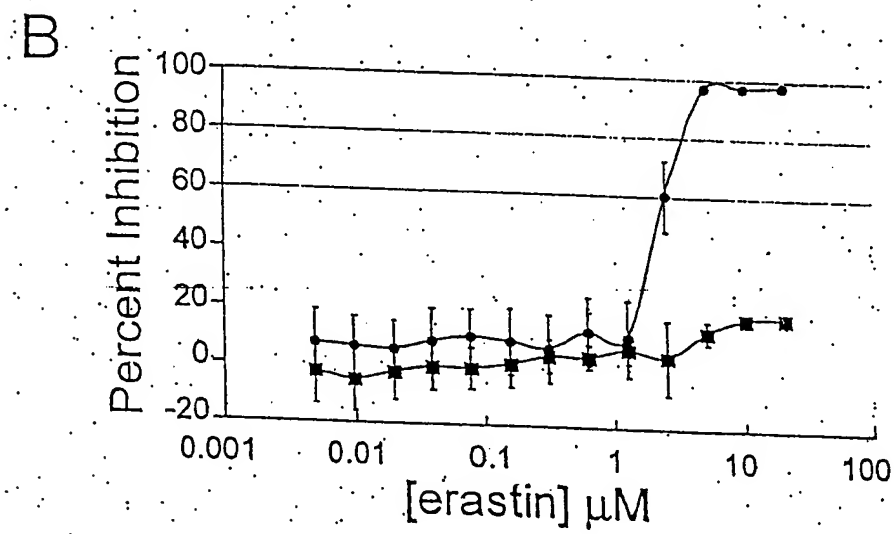

A
BJ primary
BJ-TERT
BJ-TERT/LT/ST
BJ-TERT/LT/ST/RASV12
BJ-TERT/LT/RASV12
BJ-TERT/LT/RASV12/ST
TOPIIα
eIF4E
1.0 3.4 8.1 23.2 4.4 9.5
1.0 0.8 0.8 0.8 0.9 0.9
B
BJ primary
BJ-TERT
BJ-TERT/LT/ST
BJ-TERT/LT/ST/RASV12
TOPI
eIF4E
1.0 0.4 1.3 4.3
1.0 0.8 0.9 1.0
C
Untreated
TOP1 siRNA
TOP1 siRNA
TOP1 dsDNA
lamin A/C siRNA
lamin A/C siRNA
TOPI
eIF4E
1.0 1.1 1.2 1.1 1.2 1.3
D
Number of Cells
100,000
10,000
1,000
100
0
50
100
150
TOP1 siRNA
lamin A/C siRNA
[camptothecin] ng/mL
E
Percent Inhibition
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
-10%
5.0000
1.2500
0.3120
0.0780
0.0195
0.0048
0.0012
+DMSO
+2.3 nM OA
+3.4 nM OA
+5.2 nM OA
+7.8 nM OA
[camptothecin] µg/mL
F
[okadaic acid], nM
NT NT 2.3 3.4 5.2 7.8
TOP1
eIF4E
1.0 1.5 2.3 2.8 2.2 1.9
1.0 1.0 1.1 1.1 1.1 1.0

Figure 5


A
erastin dose
100%
80%
60%
40%
20%
0%
-20%
Percent Inhibition
24
48
72
24
48
72
time (hours)
BJ-TERT/LT/ST/RAS^{V12}
BJ-TERT


B
Percent Inhibition
100
80
60
40
20
0
-20
0.001
0.01
0.1
1
10
100
[erastin] µM

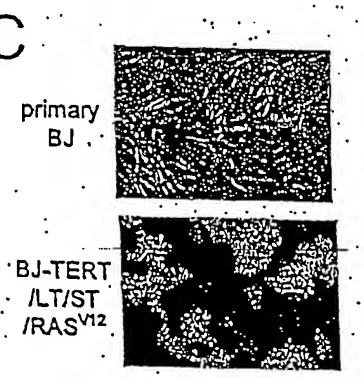

C
primary BJ
BJ-TERT /LT/ST /RAS^{V12}

Figure 6

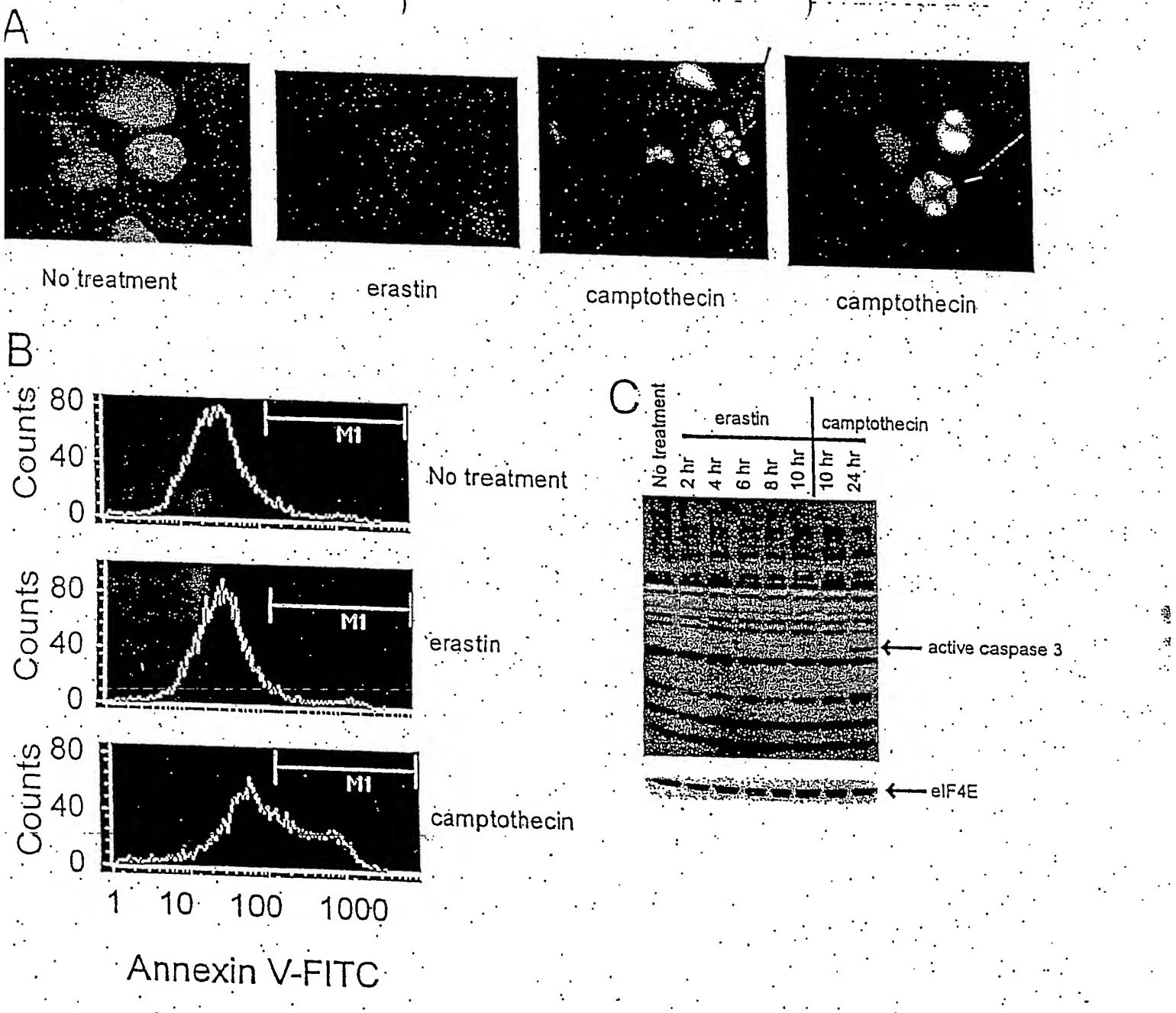
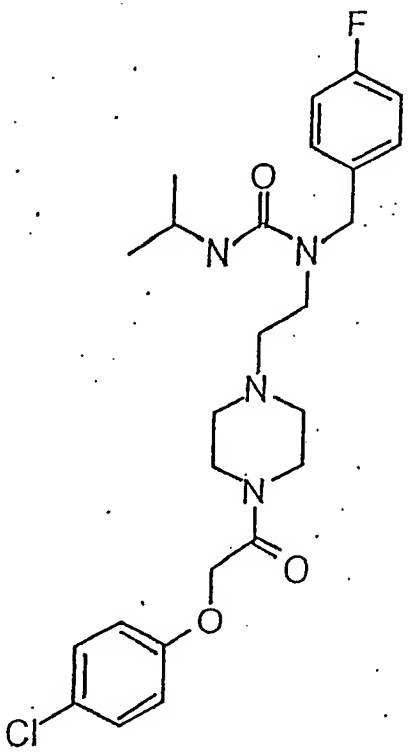

A
No treatment
erastin
camptothecin
camptothecin
B
Counts
80
40
0
M1
No treatment
erastin
camptothecin
1
10
100
1000
Annexin V-FITC
C
No treatment
erastin
camptothecin
2 hr
4 hr
6 hr
8 hr
10 hr
10 hr
24 hr
active caspase 3
eIF4E

Figure 7

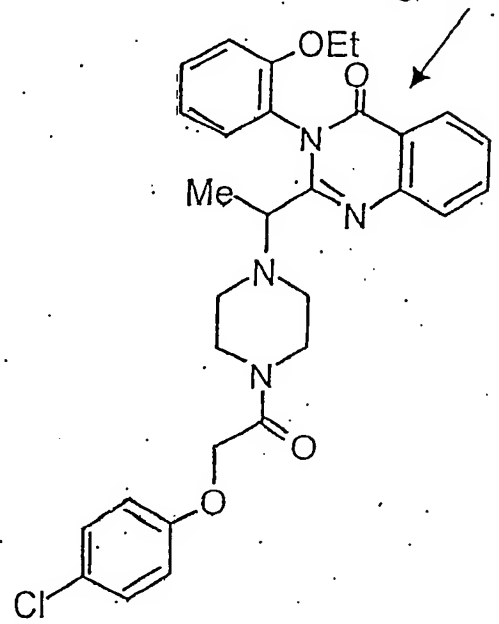
Structure Activity Relationship for Erastin

- Tested 135 analogs of erastin for activity and selectivity (tumor vs. normal)
- 134 were inactive
- 1 was active and selective, but less potent than erastin

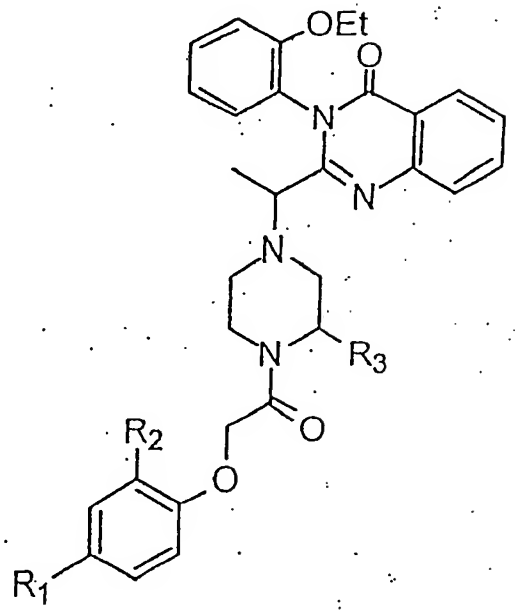


Erastin B

quinazolinone ring not required



Erastin



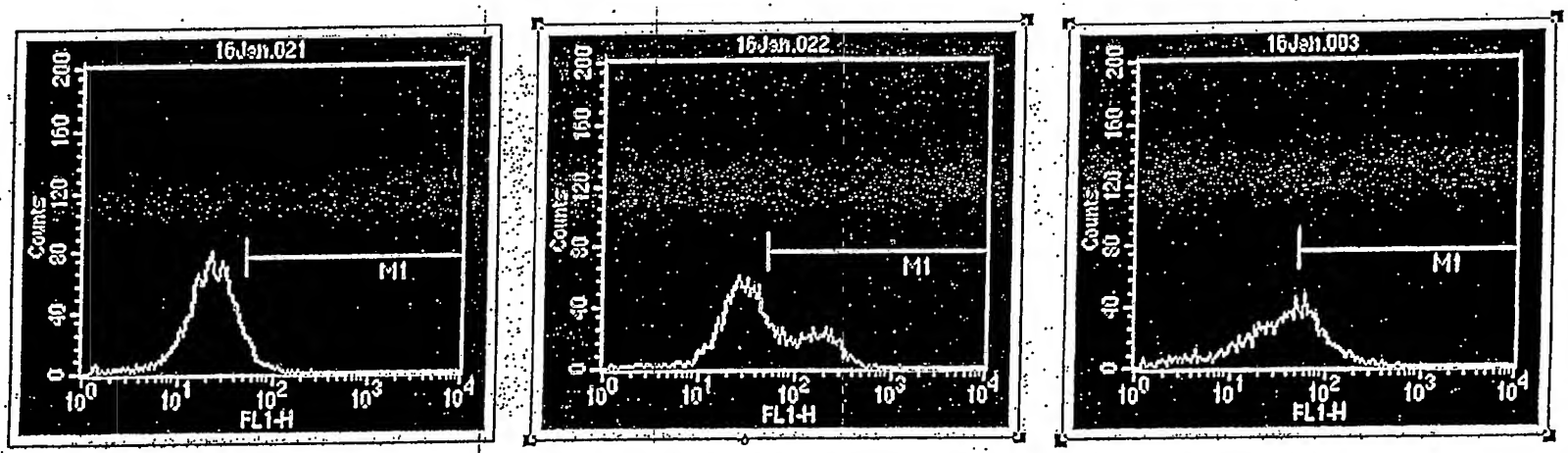
Inactive analogs

Figure 8

Nulcei Are Intact in Erastin-treated Tumor Cells

Figure 9

Erastin Induces the Formation of Reactive Oxygen Species



Untreated — Erastin-10 h treatment — H_2O_2

M1 - 5.6% — M1 - 35.1% — M1 - 37.2 %

flow cytometric analysis using dihydrodichlorofluorescein

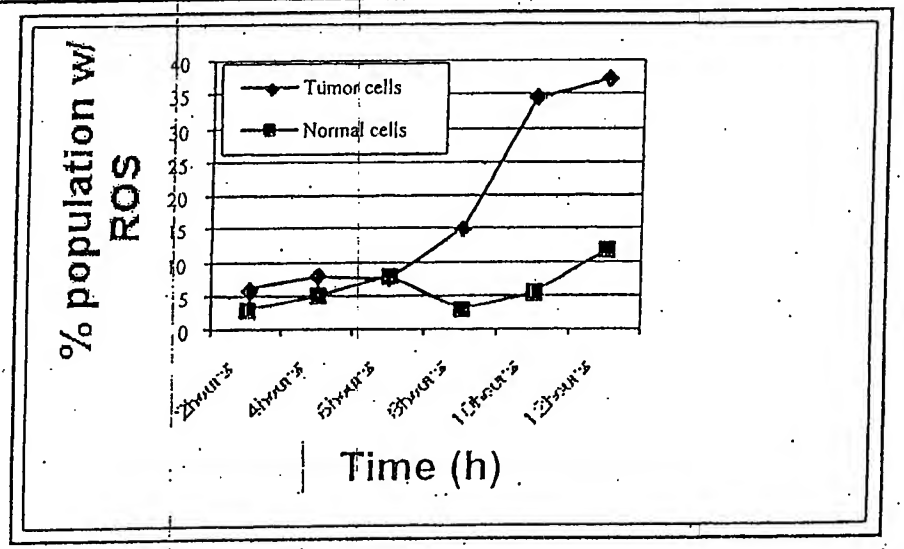
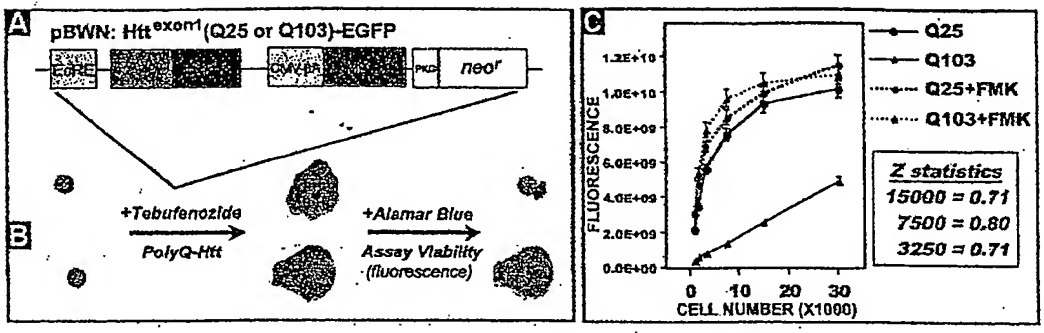
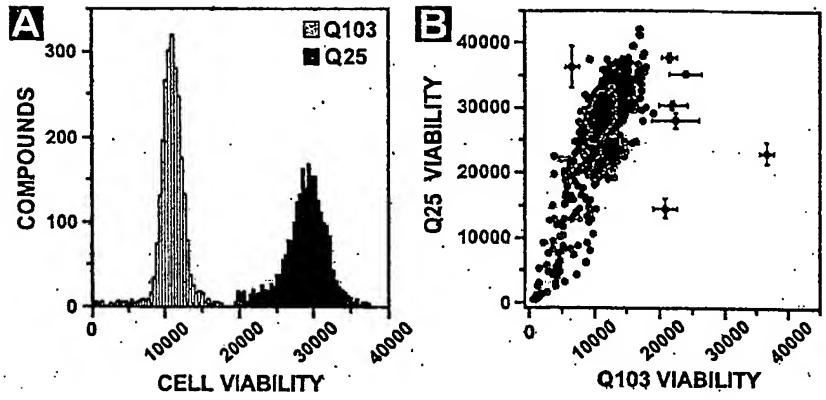


Figure 10



Modeling Htt-polyQ neurotoxicity in PC12 cells. (A) Inducible construct for production of Htt-EGFP fusion proteins. Rat neuronal PC12 cells are transfected with Htt-exon-1 constructs containing either 25 (Q25) or 103 (Q103) polyglutamine repeats (mixed CAG/CAA). (B) Cartoon of Htt-exon-1 expression in PC12 cells and screening assay for cell viability using Alamar Blue. Induction of Htt-Q103 expression leads to the formation of perinuclear cytoplasmic inclusions (or aggresomes) of the fusion protein followed by cytotoxicity after 48 hours. Expression of Htt-Q25 remains diffuse throughout the cytoplasm and is not cytotoxic. (C) Quantification of Htt-Q25 and Htt-Q103 cell viability as a measure of Alamar Blue fluorescene. Note addition of the general caspase inhibitor (BOC-D-FMK, 50 μM) rescues Htt-Q103 toxicity after a 72 hour induction with tebufenozide (Z-statistics calculated for 15000, 75000, and 3250 cells, yellow box).

Figure 11



Primary screening results of the ACL library (2036 compounds) using the Q25-Htt-exon-1 and Q103-Htt-exon-1 PC12 cell lines. The plots at left show two representations of this data set. **(A)** Histogram plot showing cell viability of Q25-Htt and Q103-Htt expressing PC12 cells after 72 hours in culture with compounds (binning interval is 400 fluorescence units). Cell viability, represented along the horizontal axis, was quantified by Alamar Blue fluorescence. **(B)** Scatter plot showing cell viability (Q25 versus Q103) following the 72 hour incubation in the presence of each compound (4 µg/ml). Color legend as follows: **Black**=2029 compounds having either no effect, cytotoxic effect, or slight rescue of cell viability; **Red**= top 6 compounds that rescued Q103-induced cell death; **Blue**= one compound that specifically enhanced Q103-mediated cytotoxicity; **Green**= overlay scatter of 400 control wells without compound (average standard deviation of control wells: Q25=3845, Q103=517). Each data point in plot (B) was calculated from an average of three replicates. The standard deviations (error bars) are shown for the 7 highlighted compounds.

Figure 12

Figure 13. Effect of cell density on coefficient of variation (CV)

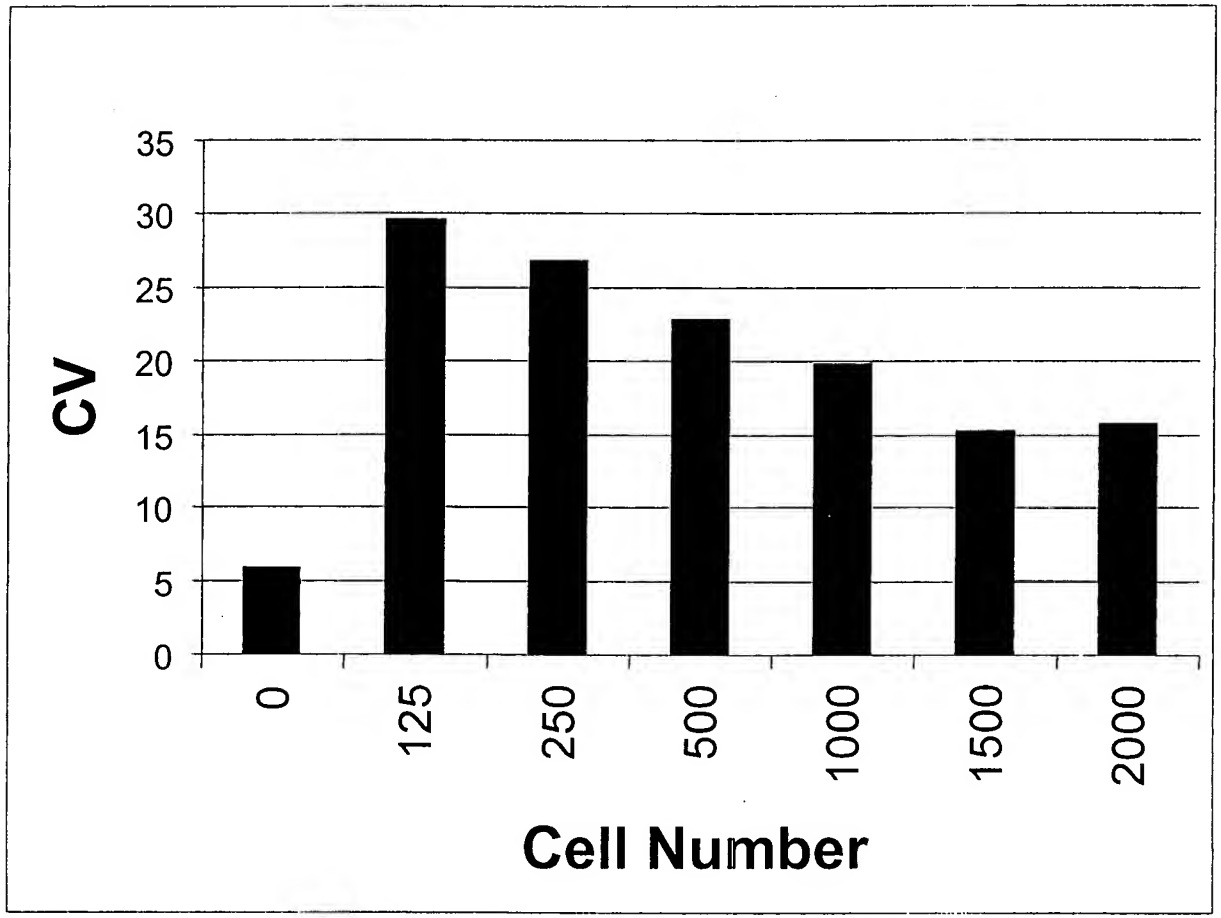


Figure 13

Figure 14. Dose-Response for Suppressor of Mutant Huntingtin-Induced Toxicity

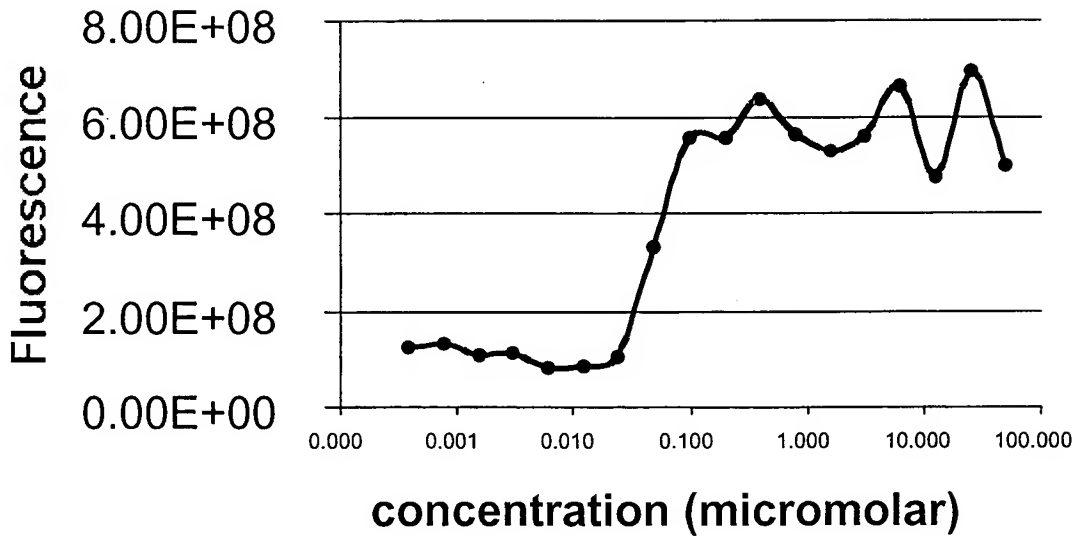
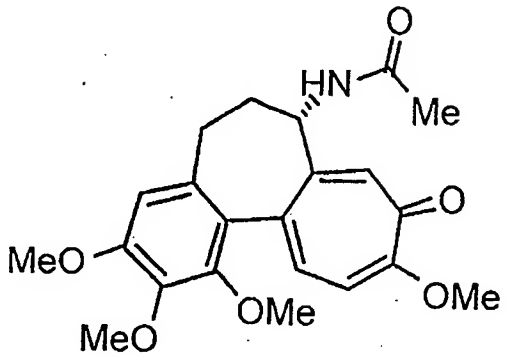
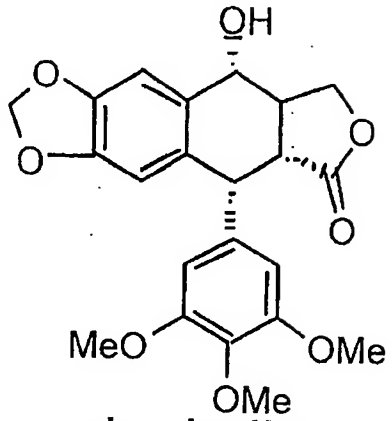


Figure 14

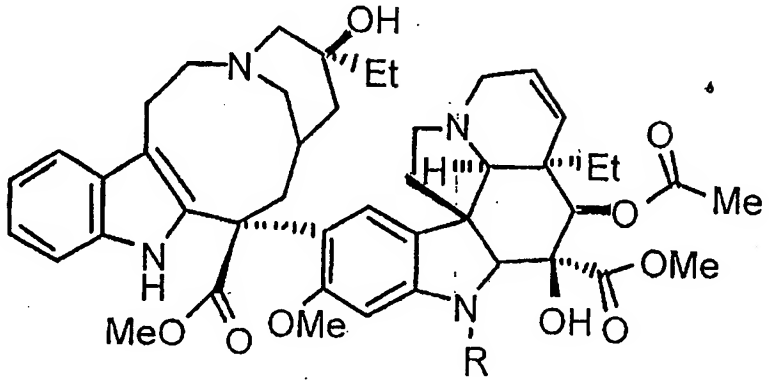
Tubulin Inhibitors Suppress Mutant Huntingtin-Induced Cell Death



colchicine



podophyllotoxin



vincristine, vinblastine

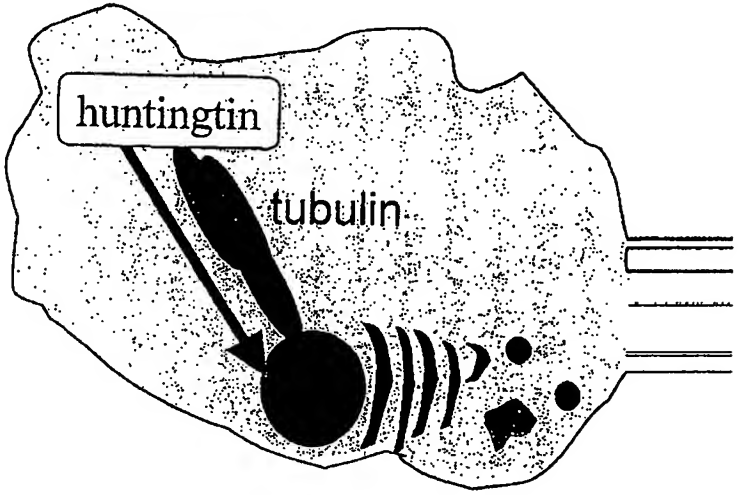


Figure 15